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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/246,451	02/09/1999	FRANCES H. ARNOLD	93731E827US1	6181

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EXAMINER

RAO, MANJUNATH N

ART UNIT PAPER NUMBER

1652

DATE MAILED: 04/08/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/246,451

Applicant(s)

ARNOLD ET AL.

Examiner

Manjunath N. Rao, Ph.D.

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 17 December 2003.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 146-154, 156-173, 175-177, 179-181, 183-185 and 187-189 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 152-156, 158, 160, 167-169, 173, 175-177, 183-185 and 189 is/are allowed.
- 6) ☒ Claim(s) 146-151, 157, 159, 161-166, 170-172, 179-181, 187 and 188 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Claims 146-154, 156-173, 175-177, 179-181, 183-185, 187-189 are currently pending in this application.

Applicants' amendments and arguments filed on 12-17-03, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 146-151, 157, 159, 161-166, 170-172, 179-181, 187-188 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a functional cytochrome P450 oxygenase variants having at least 2 to about 10 times the catalytic activity and stability of wild type cytochrome P450 oxygenase from *P.putida* and having the amino acid sequence SEQ ID NO:2 or an amino acid sequence that is at least 99% identical to SEQ ID NO:2 and comprising mutations at amino acid positions 331, 280, 242 (either individually or in combination) or having the amino acid sequence SEQ ID NO:11, 12, or 13, does not reasonably provide enablement for any such variant cytochrome P450 oxygenase comprising above specific mutations and a 90% amino acid sequence identity to SEQ ID NO: 2 or a functional variant of the above enzyme encoded by a first polynucleotide that hybridizes to a second polynucleotide

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under conditions of high stringency, wherein the second polynucleotide encodes the cytochrome P450 oxygenase variant enzyme comprising the three above specific mutations. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 146-151, 157, 159, 161-166, 170-172, 179-181, 187-188 are so broad as to encompass any variant cytochrome P450 enzyme comprising the mutations at positions and a 90% amino acid sequence identity to SEQ ID NO: 2 or a functional variant of the above enzyme encoded by a first polynucleotide that hybridizes to a second polynucleotide under conditions of high stringency, wherein the second polynucleotide encodes the cytochrome P450 oxygenase variant enzyme comprising the three above specific mutations. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of variant oxygenases broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence, and obtain the desired activity, requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant

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to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only three specific variants. It would require undue experimentation of the skilled artisan to make and use the claimed polypeptides which have the above specific mutations and a 90% amino acid sequence identity to SEQ ID NO: 2 or a functional variant of the above enzyme encoded by a first polynucleotide that hybridizes to a second polynucleotide under conditions of high stringency, wherein the second polynucleotide encodes the cytochrome P450 oxygenase variant enzyme comprising the three above specific mutations. The specification is limited to teaching use of SEQ ID NO:2 with the above specific mutations as an oxygenase with improved properties when compared to the wild type enzyme but provides no guidance with regard to the making of variants and mutants or with regard to other uses. In view of the great breadth of the claim, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in *The Protein Folding Problem and Tertiary Structure Prediction*, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by this claim.

While recombinant and mutagenesis techniques are known, and is routine in the art to screen for multiple substitutions or multiple modifications up to a certain degree, screening for variants as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in

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obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any cytochrome P450 oxygenase with 90% identity to the enzymes of SEQ ID NOS:2 because the specification does not establish: (A) regions of the protein structure which may be modified without effecting the activity; (B) the general tolerance of cytochrome P450 oxygenases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue in any cytochrome P450 oxygenase with an expectation of obtaining the desired improved biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including cytochrome P450 oxygenases with an enormous number of amino acid modifications of the cytochrome P450 oxygenase with SEQ ID NO:2. The scope of the claims must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of variant cytochrome P450 oxygenases having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office action, applicants have traversed the above rejection arguing at length that claims are indeed enabled. Applicants basically argue that it is indeed routine to screen for multiple substitutions or multiple modifications and that there is no requirement of the knowledge of which specific positions or combinations thereof of the amino acid residues in the polypeptide while using directed evolution. Applicants also argue that the tolerance to modification does not diminish with each modification where screening is performed in each step to ensure that the desired activity or stability is at least retained or improved and argue that Examiner's argument about enablement are not applicable in view of the specification and the state of art. However, in all these arguments, applicants are silent regarding undue experimentation.

Applicants argue that the quantity of experimentation is not excessive and is lessened by the high throughput screening methods and that it is routine to screen large numbers of sequences for common properties. Examiner respectfully disagrees with all the above arguments as being persuasive to overcome the above rejection. This is because, while it may be routine to screen for multiple substitutions, it is routine only up to certain extent. Furthermore, contrary to applicant's argument, polypeptides as claimed by the applicant requires a knowledge of which specific amino acid residues need to be changed. Without such knowledge, even with the high level of technical advancement in the field, such efforts would lead to undue experimentation. The amount of guidance provided by applicants is only the assay for the enzyme and not for the specific amino acid residues that can be modified to obtain a polypeptide that is at least 90% identical to SEQ ID NO:2. Examiner reiterates that applicants arguments are not persuasive to because while methods to produce variants of a known sequence such as site-specific

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mutagenesis, random mutagenesis, etc. are well known to the skilled artisan producing variants as claimed by applicants requires that one of ordinary skill in the art know or be provided with guidance for making specific modification in the amino acid sequences and the selection of which of the infinite number of variants have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification. Therefore the above rejection is maintained.

Conclusion

Claims 152-156, 158, 160, 167-169, 173, 175-177, 183-185 and 189 are allowable.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

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however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 6.30 a.m. to 3.00 p.m. If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned is 703-872-9306 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

A handwritten signature in black ink, appearing to read 'Manjunath N. Rao', with a stylized flourish at the end.

Manjunath N. Rao
April 6, 2004